LITERATURE REVIEW

1. Distribution and biology of *C. megacephala*

Chrysomya megacephala (F.), the Oriental latrine fly, is a blowfly species belonging to the Family Calliphoridae. This species is widely distributed throughout Asian regions, Australasia, the Pacific, South Africa and South America (Zumpt, 1965; Well and Kurahashi, 1994). In Thailand, it was noted as the second most abundant species collected in surveys from urban and suburban areas. Less common, only to the housefly, *Musca domestica* L., *C. megacephala* has a prevalence of 8.67-31.8% (Sucharit *et al.* 1976; Tumrasvin *et al.,* 1978; Sucharit and Tumrasvin, 1981). However, in a recent survey of the most specific flies of forensic importance, *C. megacephala* was the most abundant species found in the mountainous and downtown areas of Chiang Mai, northern Thailand (Lertthamnongtham *et al*., 2003; Sukontason *et al*., 2003).

C. megacephala has complete metamorphosis in its life cycle, with 4 stages (egg, larva, pupa and adult, respectively). Adults are relatively large, 8-11 mm in length. They have a metallic greenish blue color with purple reflections, and bright orange face. The head of the male has two compound eyes touching in the middle of the frons. The facets of the upper two-thirds are greatly enlarged and sharply demarcated from the small facets in the lower third, while the two compound eyes in females are separated by a broad frons and the upper facets are not strikingly enlarged or demarcated from the lower ones. Adults are synanthropic, commonly found near human dwellings. They are a nuisance in slaughterhouses and on meat, fish, sweets, fruit and other foodstuff in market places. Adults are strongly attracted to carrion and excrement for breeding purposes (Greenberg, 1973). One female lays 150-300 eggs in each oviposition (Zumpt, 1965). The lifespan of adults is 14-30 days (Well and Kurahashi, 1994). Nevertheless, adult longevity greatly depends on temperature and humidity. At temperatures of 25-29ºC and relative humidity of 75%, adults can survive for 54 days (90 maximum), and longer at lower humidity (Greenberg, 1973).

The egg is white, 1.5-1.6 mm in length. This stage lasts within 1 day before the larva or maggot forms with a worm-like appearance that has three instars. All three instars take ≈100 hours to develop. The larvae need high humidity and temperature for good development. They breed equally well in carrion and human feces in cesspits; however, larvae have been reported from isolated patches of cow dung. The larval stage is very active and ravenous, and competitors are crowed out. After post-feeding, the pre-pupae migrate from the breeding site to reach a dry area to pupate. After that, the skin of the third instar becomes rigid, hardened and darkened before contracting to be pupa. This lasts ≈100 hours. Then, the newly emerged fly breaks out from the pupal case. The total egg-to-adult period at room temperature is ≈8½ days (Zumpt, 1965).

2. Medical importance of *C. megacephala*

The biology and ecology of *C. megacephala* make it an ideal mechanical vector of human pathogens. Factors promoting this species as a good carrier are (i) eusynanthropy (ii) feeding habit and (iii) dispersal and great flight activity (Greenberg, 1971). Other factors such as an unhygienic sanitation, availability of food, breeding place and the proper climatic condition are also considerably involved

(Sukontason *et al*., 2000). Transmission of pathogens takes place when the adult fly makes contact with human food. Mechanical dislodgment from fly exoskeletons (e.g. hairs and bristles on the fly's body, fecal deposition and regurgitation during feeding) can transport a wide variety of bacteria, viruses, helminth eggs and protozoan cysts to human food (Kettle, 1995; Grübel *et al*., 1997; Kobayashi *et al*., 1999). Thirty species of bacteria isolated from 49 adult *C. megacephala* have been reported (Sukontason *et al*., 2000). This document mentions 3 bacterial species: *Aeromonas hydrophila*, *Edwardsiella tarda* and *Vibrio cholerae* non-01 that are the causative agent of diarrheal disease. Five other possible bacterial species of this disease are included, i.e. *Aeromonas sorbia, Citrobacter freundii, Escherichia coli, Providencia alcalifaciens* and *Pseudomonas aeruginosa*.

Of less medical concern, either small or large numbers of adult flies are pestiferous, by disturbing humans during work and at leisure. Their presence can lead to a negative psychological impact as a reminder of unhygienic conditions in the environment. Adult flies can also bother economically important animals, by disturbing their rest, which could lead to a decrease in production, thereby causing economic loss in poultry or dairy farms (WHO, 1986). On the other hand, the maggot of *C. megacephala* can induce myiasis in both humans and animals, and such cases have been reported (Zumpt, 1965; Kumarasinghe *et. al*., 2000).

3. Fly control program (WHO, 1986; 1997)

3.1 Improvement of environmental sanitation and hygiene

This approach provides a long-term result. It is more cost-effective and usually has other benefits. To retrieve good sanitation that is critical in a successful fly control program, four strategies should be applied:

• Elimination of fly breeding sites

Garbage and other organic refuse should be eliminated regularly by proper collection, storage, transportation and disposal. In the absence of a system for collection and transportation, garbage can be burnt or disposed of in a special pit covered by a fresh layer of soil to stop the flies breeding. For human excreta, installation and use of proper latrines and toilets should be carried out.

• Reduction of sources that attract flies from other areas

Attraction to waste can be prevented by cleanliness, the removal of waste and storage it under cover.

• Prevention of contact between flies and disease-causing germs

The sources of germs that infect eyes, opened sores and/or wounds are from human and animal excrement, garbage, sewage. The most important remedies for solving this problem are setting up proper latrines where flies cannot make contact with feces, preventing contact between flies and sick people and stopping access of flies to slaughterhouses and dead animals.

• Protection of food, eating utensils and people from contact with flies

Food and eating utensils can be placed in fly-proof containers or wrapping material. Screens can be used on windows. Doors can be made self-closing.

3.2 Biological control

Several species of parasites and predators are available for use in biological fly control programs. For instance, nematode *Paraiotonchium muscadomesticae* and *Steinernema feltiae* have been reported as an entomopathogen of the housefly, *Musca domestica* (Geden, 1997; Renn, 1998) along with the entomopathogenic fungus, *Entomophthora muscae* (Bellini *et al*., 1992; Krasnoff *et al*., 1995). Eight of the gram-negative bacteria, *Serratia marcescens,* caused mortality in the adult blowfly, *Lucilia sericata* (O'Callaghan *et al*., 1996). However, these investigations were carried out in the laboratory, and not in field conditions. Studies conducted to date have not yet shown a significant effect on vertebrates and some none-target arthropods from these entomopathogens or their toxins.

3.3 Methods of killing flies directly: physical or chemical methods

Physical means (e.g. traps, fly swats, electrocuting grids) are easy to use and avoid the problem of insecticide resistance, but they are not effective when fly densities are high. They are particularly suitable for small-scale use in hospitals, offices, hotels and supermarkets for instance.

Insecticides can temporarily lead to very quick control, which is essential during outbreaks of cholera, dysentery or trachoma. The WHO (1986) recommended six main types of application for chemical fly control, as follows:

- Larvicides for treating breeding places
- Residual sprays for applying to resting-sites and other surfaces
- Impregnated strips, cords, etc. for introducing toxic resting-sites
- Toxic baits

- Space sprays and directs spraying of fly aggregations indoors and outdoors
- Fumigation.

3.4 <u>Mechanism of action and neurotoxic effects of common insecticides</u>

Several organophosphorus compounds and carbamates are used as insecticides. Their acute toxic effect on the central and peripheral nervous system is due to the inhibition of acetylcholinesterase at the nerve endings, which causes an accumulation of acetylcholine and consequently overstimulation of the nicotinic and muscarinic receptors. Although long-term, mild neurobehavioural changes of questionable significance have been reported in some instances, recovery from the cholinergic syndrome appears to be complete, unless lesions develop in the central nervous system as a consequence of either convulsions or anoxia. The toxicological relevance of these interactions is still unclear. Certain organophosphorus compounds induced delayed polyneuropathy, which develops 2-5 weeks after an acute poisoning. The molecular target is believed to be neuropathy target esterase (Moretto, 1998).

Administration of deltamethrin markedly increased the wet weight of the hippocampus and pons medulla region without much affect on the weight of the frontal cortex, corpus striatum, hypothalamus, and cerebellum. A significant increase in the activity of monoamine oxidase was observed in the frontal cortex, hippocampus, and cerebellum, and acetylcholinesterase activity was strikingly increased in the frontal cortex, corpus striatum, hippocampus, cerebellum, and pons medulla. Deltamethrin significantly increased the spontaneous locomotor activity and aggressive behavior. Significant neurochemical and neuromorphological changes may culminate in perturbed synaptic function following deltamethrin exposure in rats (Husain *et al* 1996).

4. Neuroanatomical structure of the fly brain (Chapman, 1998; Romoser and Stoffolano, 1998)

The brain or supra-esophageal ganglion is the most well-known vital organ of the insect nervous system. It is a very complex structure, apparently consisting of three parts: the protocerebrum, deutocerebrum and tritocerebrum (Figure 1).

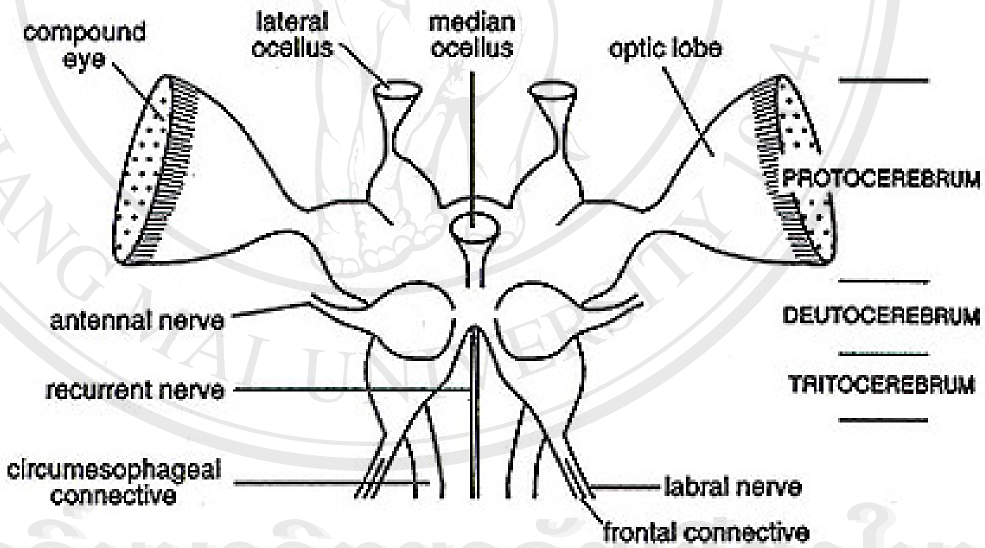

Figure 1 Frontal view diagram of insect brain (Chapman, 1998; p.552).

The protocerebrum is regarded as the most important portion. Several distinct cell masses and regions of the neuropil have been identified. The *optic lobes*, associated with the compound eyes, comprise three neuropils and associated perikarya. The *ocellar centers* associate with the bases of the nerve from the dorsal ocelli. The *central body* or *central complex* connects the two lobes of the protocerebrum and is situated in the center of the protocerebrum dorsal to the esophagus. The *protocerebral bridge* is the mass of neuropil located medially. It is associated with axons from many parts of the brain, except the corpora pedunculata. The *pars intercerebralis* is located in the dorsal median region above the protocerebral bridge and central body. It contains two groups of neurosecretory cells that transport neurohormones, ecdysiotropin or prothoracicotropic hormone (PTTH), to the corpus cardiaca. This hormone directly stimulates the endocrine organs of the fly to secrete other essential hormones for control of the overall physiological or behavioral process. The *corpora pedunculata* or *mushroom bodies* contain interneurons and terminal portions of axons that enter from perikarya located in other parts of the brain.

The deutocerebrum contains the *antennal* or *olfactory lobes*, which receive both sensory and motor axons from the antennae. Each antennal lobe maintains spheroidal collections of neuropil called glomeruli. The antennal lobes are connected to one another by a central fiber tract. The tracts of olfactory fibers connect the antennal lobes and the corpora pedunculata of the protocerebrum.

The tritocerebrum links the brain to the stomatogastric nervous system via the frontal ganglion and to the ventral chain of the ganglia (beginning with the subesophageal ganglion) via the circumesophageal connectives. The tritocerebrum also receives nerves from the labrum and possibly sensory fibers from the head capsule.

There are several neuroanatomical studies on the brain of other flies, such as the housefly, fleshfly (*Sarcophaga bullata*), fruitfly (*Drosophila melanogaster*) or honey bee (*Apis mellifera*), but none for the blowfly, *C. megacephala.*

The entire brain of the honey bee, *A. mellifera,* has been studied for the background requirement of the fly brain's fiber tracts and neuropils. The vertical, sagittal and horizontal sections of the whole brain were stained at various depths by silver-intensified- and Cobalt-stain. The neuropils in all lobes of the brain stained with Golgi stain have also been demonstrated (Mobbs, 1985).

Jasanoff and Sun (2002) reported the comparison between near-cellular-resolution magnetic resonance (MR) images and histology-based anatomical structures of an unanesthetized fleshfly of *S. bullata*. The authors implied that the MR microscopy faithfully represents patterns of nervous tissue and allows distinct brain regions to be clearly identified. In addition, they suggested that the challenges of noninvasive *in vivo* MR microscopy should be useful in a system amenable to studying the brain structure.

In 2002, the wild-type Canton S *D. melanogaster* brain was chosen as the reference to study standard brain morphology using the combination of immunochemistry, high-resolution 3D confocal microscopy, and advanced graphic computing. These approaches led to a convergence of neuroanatomical data from different sources and resulted in a new type of atlas (database) combining structural, developmental and molecular information (Rein *et al*., 2002).

5. Biology of aging

All multi-cellular organisms undergo change with time (Sohal and Weindruch, 1996). The onset of growth and development leads to reproductive competence and propagation of the species. With time, organism aging leads to death as a finale. While our knowledge and definitions of growth and reproduction are firmly established, the concept of aging is not so well understood.

Many scientists have defined the term of aging such as Comfort, Harman and Buss. In Comfort's view, aging is a result of progressive loss of physiological function that culminates in death (Helfand and Inouye, 2003). Harman has defined aging as the accumulation of changes responsible for the sequential alterations that accompany advancing age, and the associated progressive increase in the chance of disease and death (Harman, 1991; Wickens, 2001). Recently, Buss divided the aging processes into primary and secondary aging. In his view, primary aging is intrinsic to the organism, and the detrimental factors are determined by inherent or hereditary influences (Wong, 2001). On the other hand, secondary aging is cause by deleterious or hostile factors in the environment. However, due to the complexity of the aging process, these descriptive definitions of aging are always far from satisfactory.

Troen (2003) suggested that the terms 'aging' and 'senescence' are often used interchangeably. Both are characterized by progressive changes in the tissues or organs of the body, leading to a decline in function and death. In humans, some of the common features associated with aging include loss of skin elasticity, loss of hair, decline in renal function, development of atherosclerosis and coronary artery disease. Most of these recognizable features occur after reproductive activity has ceased and the term 'senescence' is often used to describe this period, which is associated with degenerative changes related to the passage of time. ‘Normal’ aging involves inexorable and universal physiological changes, whereas ‘usual’ aging includes age-related diseases. For example, menopause represents aspects of normal aging. In contrast, coronary artery disease is an example of usual aging and is not found in all older persons. Troen also summarized the five common characteristics of aging in mammals as follows:

- Increased mortality with age after maturation
- Changes in biochemical composition in tissues with age
- Progressive decrease in physiological capacity with age
- Reduced ability to respond adaptively to environmental stimuli with age
- Increased susceptibility and vulnerability to disease.

Regarding the question of how aging can be measured, two fundamental parameters apply: (i) the maximum lifespan potential, and (ii) the expectation of life or average lifespan. The maximum lifespan potential represents the member(s) of the population or species who lived longest and the average lifespan are represented by the age at which 50% of given population survive. The average lifespan of humans has increased dramatically over time, yet the maximum lifespan potential has remained approximately constant and is usually stated to be 90-100 years. Throughout most of recorded human history, socioeconomic and nutritional status have been strongly associated with life expectancy and, along with disease, resulted in significant variations in the lifespan of individuals.

Romoser and Stofolano (1998) claimed that the aging process of insects is an appropriate way to complete a discussion on postembryonic morphogenesis, since it refers to all the changes in structure and function that decrease its capacity for survival and finally leads to death. They also cited theories on the causes of senescence that are based in two areas; heredity and environment. Probably, in the majority of situations, both heredity and environment act together to produce the observed senescence.

Heredity factors are as follows:

- Aging as the result of programmed cessation of growth
- Programmed retardation and/or failure of some substance necessary for maintenance of the nonsenescing state
- Depletion of DNA, RNA, enzymes and other substances essential for cell function
- Scheduled production of an aging substance and accumulation of materials that may become harmful to an organism in time.

Environmental factors are as follows:

- Cumulative radiation (ionizing, infrared, etc.) effect
- Cumulative effects from parasitic invasion
- Cumulative effects of physical insults such as extremes in temperature or mechanical injury.

Among insect species, life spans are quite variable. Differences in mean lifespan have been found among various genetic strains, populations with different diets and populations exposed to different temperature regimes.

6. The behavioral changes in the senescent fly (Romoser and Stoffolana, 1998)

Flies are continually attacked with multi-innumerable stimuli in their environment. In order to survive, they must respond to the right stimuli and do the right thing at the right time: escape, locate a food source, feed, locate a mate, copulate, oviposit, migrate, become dormancy and so on. The behavior of adults has been noted to associate with their age. In the observation of adult *C. megacephala* in the insectary at Department of Parasitology, Faculty of Medicine, Chiang Mai University, young flies are active in both movement and mating; while old ones or those aged >30 days, are sluggish and/or clumsy. Moreover, decreased oviposition in old females has been observed.

7. The pathological change in the brain of the senescent flies

Sohal and Sharma (1972) studied the age-related changes in the fine structure and number of neurons in the brain of the senile male housefly. The neuronal populations in 3-day-old flies and those of 30 days old showed no statistically significant difference in number. However, the neurons of old aged flies exhibited several significant deteriorating changes in fine structure. These were loss of ribosomes, focal cytoplasmic degeneration and accumulation of dense residual bodies. Senescence-accelerated fruit fly is a valuable tool for investigating and understanding the molecular mechanisms of neurodegenerative diseases, because of its highly evolved nervous system, amenability to genetic analysis, and the full genomic sequence available. A systemic screen for short-life mutants was carried out by ethyl methane sulfonate (EMS) and P-element insertion. The brain of short-life mutants and the reference line were examined at various ages by Toluidine blue staining of semi-thin plastic sections for light microscopy and electron microscopy of ultrathin sections. Twenty-one neurodegenerative mutants including *bubblegum*, *spongecake*, and *eggroll* were isolated and named by virtue of their brain lesions. Each mutant had a distinct age-associated histological pattern of degeneration in specific regions of the brain. Degeneration occurs in the lamina and retina region in *bubblegum*. In *spongecake*, vacuolization can only be seen in the optic lobe, especially in the medulla region. Multilamellated inclusions are widespread in the brain of *eggroll* (Min, 2001).

OBJECTIVES OF THIS STUDY

1. To investigate the structural and ultrastructural changes of the fly brain at a young and old age.

2. To determine the neuron loss in each area of the fly brain and compare it between old and young flies.